

## **Supplemental Material to:**

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**Adaptations of placental and cord blood ABCA1 DNA methylation profile to maternal metabolic status**

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## SUPPLEMENTAL MATERIAL

**Supplementary Table 1.** PCR and pyrosequencing primers for *ABCA1* gene CpG island locus amplification

<b>Amplified region</b>	<b>PCR and pyrosequencing primers</b>	<b>Length (bp)</b>
<i>ABCA1</i> -A (8 CpGs)	F: 5'bio-GGGTGGAGGGTATAGTAGGT-3' R: 5'-AACAAATTCCACTAATACCCTTAACT-3'; Seq: 5'-AACAAATTCCACTAATACCCTTAACT-3'	161 bp
<i>ABCA1</i> -B (4 CpGs)	F: 5'bio-GGGTGGGGAGAGTTGTAG-3' R: 5'-CCCAAATCAAAACAAACCAACTTCTTA-3' Seq: 5'-CCAACCTCAAAACCCT-3'	163 bp
<i>ABCA1</i> -C (4 CpGs)	F: 5'bio-TTGATTTGGGAATTGGTTATATGTT-3' R: 5'-AACAAAAACCCAAAACCTCTAATAA-3' Seq: 5'-AAAACACCTCCAACCT-3'	135 bp
<i>ABCA1</i> -D (6 CpGs)	F: 5'-AATTTGTTGGTTAGGTTGGTT-3' R: 5'bio-AAAAAAAAAATTAACACCCACCTCC-3' Seq: 5'-AGTGTGGGATTATAGG-3'	180 bp
USF-bio USR	5'Bio-GTGACGTACTAGCAACG-3' 5'-TAGCAGGATACGACTATC-3'	-

F; Forward. R; Reverse. Seq; Sequencing

Primers were designed using the PyroMark Assay Design v2.0.1.15 software (Qiagen). Sequences are shown for the standard format with biotin attached to the specific primer. A non-human universal tail was attached to the 5' ends of both specific primers in order to decrease cost and add flexibility to the pyrosequencing method as previously described (42). Following universal tails were attached to the 5'-ends of the specific primers for the universal primer approach: 5'-GTGACGTACTAGCAACG-3' (USF) replaced biotin in the biotinylated primer, and 5'-TAGCAGGATACGACTATC-3' (USR) extended the non-biotinylated primers. Only the universal primer USF was synthesized as a biotinylated primer.

**Supplementary Table 2.** Pearson's correlation coefficients obtained between maternal characteristics and *ABCA1* DNA methylation of placenta and cord blood.

Maternal characteristics	Maternal side of placenta		fetal side of placenta		Mean cord blood <i>ABCA1</i> DNA methylation
	<i>ABCA1</i> -CpG5	<i>ABCA1</i> -CpG6	<i>ABCA1</i> -CpG5	<i>ABCA1</i> -CpG6	
1 <sup>st</sup> trimester - Age (years)	<i>r</i> =-0.01 NS	<i>r</i> =-0.05 NS	<i>r</i> =0.11 NS	<i>r</i> =-0.17 <i>p</i> =0.10	<i>r</i> =0.10 NS
1 <sup>st</sup> trimester- BMI (kg/m <sup>2</sup> )	<i>r</i> =0.03 NS	<i>r</i> =0.06 NS	<i>r</i> =0.01 NS	<i>r</i> =0.16 <i>p</i> =0.10	<i>r</i> =-0.12 NS
1 <sup>st</sup> trimester- Waist circumference (cm)	<i>r</i> =0.06 NS	<i>r</i> =0.02 NS	<i>r</i> =0.04 NS	<i>r</i> =0.15 NS	<i>r</i> =-0.12 NS
1 <sup>st</sup> trimester - Fasting glucose (mmol/L)	<i>r</i> =-0.02 NS	<i>r</i> =0.07 NS	<i>r</i> =-0.18 <i>p</i> =0.08	<i>r</i> =-0.06 NS	<i>r</i> =0.16 NS
1 <sup>st</sup> trimester - Fasting insulin (mU/L) <sup>a</sup>	<i>r</i> =0.03 NS	<i>r</i> =0.05 NS	<i>r</i> =-0.15 NS	<i>r</i> =-0.08 NS	<i>r</i> =-0.05 NS
1 <sup>st</sup> trimester – HOMA-IR <sup>a</sup>	<i>r</i> =0.04 NS	<i>r</i> =0.03 NS	<i>r</i> =-0.16 NS	<i>r</i> =-0.08 NS	<i>r</i> =-0.06 NS
1 <sup>st</sup> trimester - Total cholesterol (mmol/L)	<i>r</i> =-0.09 NS	<i>r</i> =-0.07 NS	<i>r</i> =-0.03 NS	<i>r</i> =0.05 NS	<i>r</i> =-0.01 NS
1 <sup>st</sup> trimester – HDL-C (mmol/L)	<b><i>r</i>=-0.21</b> <b><i>p</i>=0.04</b>	<b><i>r</i>=-0.24</b> <b><i>p</i>=0.02</b>	<i>r</i> =0.02 NS	<i>r</i> =0.03 NS	<i>r</i> =0.07 NS
1 <sup>st</sup> trimester – LDL-C (mmol/L)	<i>r</i> =-0.02 NS	<i>r</i> =0.01 NS	<i>r</i> =-0.03 NS	<i>r</i> =0.04 NS	<i>r</i> =0.05 NS
1 <sup>st</sup> trimester - TG (mmol/L) <sup>a</sup>	<i>r</i> =0.15 NS	<i>r</i> =0.11 NS	<i>r</i> =0.17 <i>p</i> =0.09	<i>r</i> =0.06 NS	<b><i>r</i>=-0.24</b> <b><i>p</i>=0.03</b>
2 <sup>nd</sup> trimester - 2h-post-OGTT glucose concentration (mmol/L)	<b><i>r</i>=0.25</b> <b><i>p</i>=0.02</b>	<i>r</i> =0.15 NS	<i>r</i> =-0.01 NS	<i>r</i> =-0.04 NS	<b><i>r</i>=-0.26</b> <b><i>p</i>=0.02</b>
Weight gain between 1 <sup>st</sup> and 3 <sup>rd</sup> trimester (% of initial weight) <sup>b</sup>	<b><i>r</i>=-0.26</b> <b><i>p</i>=0.02</b>	<i>r</i> =-0.01 NS	<i>r</i> =-0.16 NS	<i>r</i> =-0.01 NS	<i>r</i> =0.14 NS
Mean blood <i>ABCA1</i> DNA methylation (%)	<i>r</i> =-0.11 NS	<i>r</i> =-0.04 NS	<i>r</i> =0.07 NS	<i>r</i> =-0.01 NS	<b><i>r</i>=0.51</b> <b><i>p</i>&lt;0.001</b>

Correlation coefficients (*r*) and *p*-values were obtained after correction for these potential confounding factors when appropriate : maternal age, BMI, fasting triglyceridemia at first trimester, weight gain between first and third trimester and previous history of GDM.

<sup>a</sup>Correlation coefficients and *p*-values obtained after log10-transformation of the variable.

<sup>b</sup>*n*=95

NS = *p*>0.10

**Supplementary Table 3.** Pearson's correlation coefficients obtained between fetal characteristics and *ABCA1* DNA methylation of placenta and cord blood.

Fetal characteristics	Maternal side of placenta		Fetal side of placenta		Mean cord blood <i>ABCA1</i> DNA methylation
	<i>ABCA1</i> -CpG5	<i>ABCA1</i> -CpG6	<i>ABCA1</i> -CpG5	<i>ABCA1</i> -CpG6	
Gestational age (weeks)	$r=-0.13$ NS	$r=0.19$ $p=0.06$	$r=0.06$ NS	$r=-0.07$ NS	$r=-0.03$ NS
Birth weight (kg)	$r=0.06$ NS	$r=0.09$ NS	$r=0.16$ NS	$r=0.10$ NS	$r=0.12$ NS
Placenta weight (g) <sup>a</sup>	$r=0.04$ NS	$r=0.06$ NS	$r=0.19$ $p=0.07$	$r=0.13$ NS	<b><math>r=0.29</math></b> <b><math>p=0.01</math></b>
Fetal-placental weight ratio <sup>a</sup>	$r=-0.06$ NS	$r=-0.03$ NS	$r=-0.16$ NS	$r=-0.11$ NS	<b><math>r=-0.25</math></b> <b><math>p=0.03</math></b>
Cord blood total cholesterol (mmol/L) <sup>b</sup>	$r=-0.01$ NS	$r=-0.08$ NS	$r=0.03$ NS	$r=0.10$ NS	$r=-0.10$ NS
Cord blood LDL-C (mmol/L) <sup>b,c</sup>	$r=0.04$ NS	$r=-0.11$ NS	$r=0.04$ NS	$r=0.08$ NS	$r=-0.05$ NS
Cord blood HDL-C (mmol/L) <sup>b</sup>	$r=-0.05$ NS	$r=-0.10$ NS	$r=0.19$ $p=0.09$	$r=0.12$ NS	$r=-0.11$ NS
Cord blood TG (mmol/L) <sup>b,c</sup>	$r=0.01$ NS	$r=0.07$ NS	<b><math>r=-0.28</math></b> <b><math>p=0.01</math></b>	$r=-0.14$ NS	$r=-0.13$ NS
Cord blood c-peptides (pmol/L) <sup>d</sup>	$r=0.02$ NS	$r=0.13$ NS	$r=-0.12$ NS	$r=-0.06$ NS	$r=-0.15$ NS

Correlation coefficients ( $r$ ) and  $p$ -values were obtained after correction for these potential confounding factors when appropriate: newborn sex, gestational and maternal age, BMI, fasting triglyceridemia at first trimester, weight gain between first and third trimester and previous history of GDM.

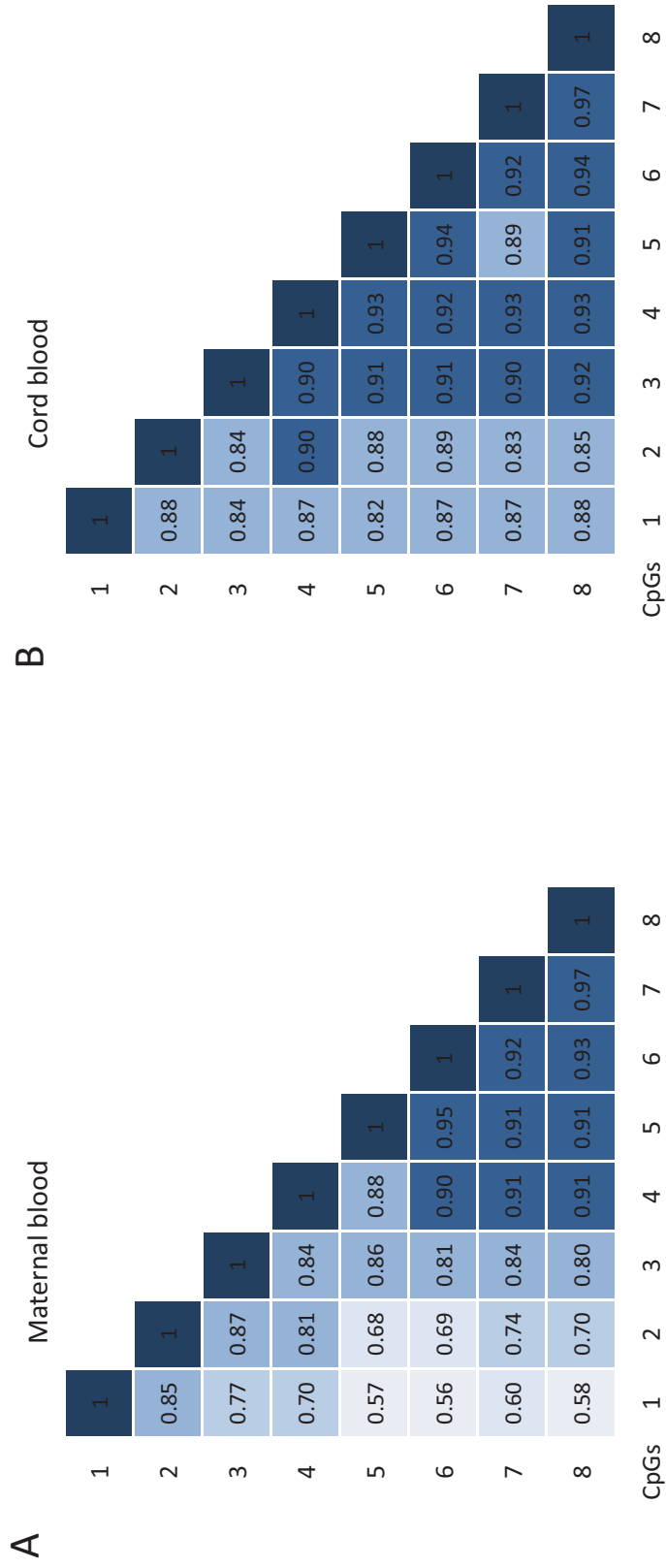
<sup>a</sup> $n=91$

<sup>b</sup> $n=87$

<sup>c</sup>Correlation coefficients and  $p$ -values obtained after log<sub>10</sub>-transformation of the variable

<sup>d</sup> $n=63$

NS =  $p>0.10$



**Supplementary Figure 1: Pearson correlation coefficients between CpGs at ABCA1-A locus in maternal (A) and cord blood (B)**